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Genetic variability and conservation of the endangered Pannonian root vole in fragmented habitats of an agricultural landscape

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Abstract

The distribution of the endangered glacial relict subspecies, the Pannonian root vole *Alexandromys oeconomus mehelyi* Éhik, 1928, is restricted to scattered localities in south-western Slovakia, which belong to the north-eastern zone of its range. Human-induced changes and fragmentation of the landscape have led to the gradual loss of suitable habitats and threaten its long-term survival. The study area in the Danubian Lowland is characterised by small habitat fragments and temporal fluctuations of the habitat area. Root voles were sampled at nine sites to study the level of genetic variability and structure of local subpopulations by scoring 13 microsatellite loci in 69 individuals. Genetic differentiation varied amongst local populations and we did not find a significant isolation-by-distance pattern. Bayesian clustering analysis suggested that dispersal effectively prevents marked genetic subdivision between studied habitat fragments. Significant pairwise differentiation between some subpopulations, however, may be the result of putatively suppressed gene flow. Low genetic diversity in the recent populations probably reflects the isolated location of the study area in the agricultural landscape, suggesting that long-term survival may not be assured. In order to maintain genetic diversity, it is essential to preserve (or even restore) habitats and ensure the possibility of gene flow; habitat protection is, therefore, recommended. Continuous assessment is necessary for effective conservation management and to predict the long-term survival chances of the Pannonian root vole in the study area.

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Keywords
Alexandromys oeconomus mehelyi, Danubian Lowland, landscape change, microsatellite analysis

Introduction

The root vole *Alexandromys* (*Microtus*) *oeconomus* (Pallas, 1776) is the only extant Holarctic species of the Microtini tribe with twenty-five known subspecies distributed in the Northern Hemisphere (Pardiñas et al. 2017). The widespread species is categorised as 'Least Concern' in the IUCN Red List of Threatened Species (Linzey et al. 2016).

As a result of glacial and postglacial changes in its distribution range, the root vole is currently represented by three subspecies in Western and Central Europe (Musser and Carleton 2005; Pardiñas et al. 2017), *A. oeconomus stimmingi* (Nehring, 1899) (Nehring 1899), *A. oeconomus arenicola* (de Sélys-Longchamps, 1841) (de Sélys-Longchamps 1841) and *A. oeconomus mehelyi* Éhik, 1928 (Éhik 1928), all of which belong to the Central European phylogroup (Brunhoff et al. 2003). As a protected species of the European fauna, the root vole is included in Appendix III of the Bern Convention (Council of Europe 1982). Two subspecies, *A. o. mehelyi* and *A. o. arenicola*, are endangered and need a feasible conservation action plan with special management strategy to maintain populations (Pardiñas et al. 2017). The Pannonian root vole *A. o. mehelyi*, an isolated subspecies present in the Carpathian Basin, occurs in the southern part of Slovakia in the Danubian Lowland (Miklós et al. 2014; Ambros et al. 2016), locally in north-eastern Austria (Thissen et al. 2015a) and in some isolated localities in Hungary (Horváth and Herczeg 2013; Lanszki et al. 2015; Kalmár and Riezing 2017). This subspecies is considered to be a glacial relict in Central-Europe (Brunhoff et al. 2003) with populations inhabiting refugial areas including freshwater marshes, swamps, floodplains or watersides (Baláž and Fraňová 2013; Kalmár and Riezing 2017). Based on intensive research in the past, all studies defined the suitable habitats of the Pannonian root vole as wet sites with dense vegetation cover, typically dominated by sedge (*Carex* sp.) or hummocks of sedge and common reed (*Phragmites australis*) mosaics (Kratochvíl and Rosický 1955; Stollmann and Ambros 1998; Hulejová Sládkovičová et al. 2016). Due to its special habitat requirements and rarity, apart from some larger known populations, it is common that merely one or a few specimens are found sporadically or only indirect evidence indicates its occurrence (Miklós et al. 2011; Purger 2014; Thissen et al. 2015a, b). The subspecies is sensitive and exhibits rapid response to natural and anthropogenic disturbances, especially to their additive effects and it was shown that individuals could cross barriers to reach suitable areas (Horváth and Herczeg 2013). Habitat loss, fragmentation and degradation due to changes in land-use, like drainage or mowing, are amongst the well-known risk factors that threaten the Panonian root vole (Thissen et al. 2015a; Gubányi et al. 2009). As this rare, habitat specialist subspecies is present exclusively in scattered populations in these remnant habitats, it appears amongst the priority species of communities in Annexes II and IV of the Habitats Directive (European Commission 1992).

The distribution of Pannonian root vole in the territory of Slovakia is determined by the change of the landscape and its natural conditions in the postglacial era. Over the past 130 years, the Danubian Lowland in southern Slovakia has experienced significant landscape modifications, such as extensive flood protection interventions and construction of a channel network in agricultural lands. As a consequence, habitats preferred by the root vole are currently fragmented and isolated by agricultural lands in southern Slovakia (Ambros et al. 2016). Furthermore, flooding events, the oscillation of ground water level and dry periods have a substantial effect on the connectivity between remnant patches of suitable habitat, determining gene flow amongst them. Landscape fragmentation and loss of habitats represent a significant risk factor in the Danubian Lowland for this rare subspecies which occurs only in scattered populations in south-western Slovakia, thus, the Pannonian root vole is strictly protected in the country (Miklós et al. 2014; Ambros et al. 2016).

Habitat loss and fragmentation caused by landscape modification affect wildlife populations worldwide (Wilson et al. 2016). The negative impact of edge effects, higher sensitivity to environmental and demographic stochasticity and the change of different characteristics of small, fragmented populations in reduced habitats may lead to increased risk of local extinction (Willi and Hoffmann 2009). Simultaneously, as the isolation of remnant patches becomes larger, dispersal and thus gene flow amongst local populations may become hampered (Storfer et al. 2010). Consequently, subdivided and isolated populations undergo changes in their genetic structure and variability (Lino et al. 2019).

The response of small mammalian populations to habitat fragmentation has been widely studied (e.g. Kozakiewicz et al. 1999; Mortelliti et al. 2010). Wetland and marshland associated species are particularly threatened by the negative effects of fragmentation (Rushton et al. 2000; Pita et al. 2010), as the available habitats are already fragmented and isolated to varying degrees as a result of human activities, such as infrastructure development, water management or agricultural drainage (Čížková et al. 2013). The consequences of habitat fragmentation on genetic diversity in subdivided small mammal populations have been in the focus of comprehensive research (e.g. Gaines et al. 1997; Fietz et al. 2014). A number of papers aimed to study Microtine rodents as appropriate model organisms (Redeker et al. 2006; Marchi et al. 2013) in the investigation of habitat fragmentation which poses substantial threat to endemic and relict vole species or subspecies that are especially sensitive to its negative effects (Buzan et al. 2010; Pita et al. 2014). Rare species and subspecies, including voles inhabiting wetlands, have been the subject of studies that investigated the genetic characteristics of populations occurring in fragmented or isolated habitats in the Nearctic (List et al. 2010; Neuwald 2010; Parmenter et al. 2015) and Palearctic regions (Van De Zande et al. 2000; Telfer et al. 2003; Centeno-Cuadros et al. 2011) and, based on the levels of genetic diversity and revealed genetic patterns, many authors stressed implications or proposed measures for conservation.

To ensure the viability of root vole populations or subpopulations, it is reasonable to study genetic variability and genetic structure as factors affecting the adaptive traits

and future persistence of populations. Wetland habitats experienced fragmentation and significant shrinkage in south-western Slovakia and only a few habitat fragments are left for root voles. In the present survey, we used microsatellite analyses to study the genetic diversity and structure of Pannonian root vole subpopulations inhabiting remnant habitat fragments in the Danubian Lowland, where the greatest threat to their long-term survival is habitat loss, fragmentation and degradation (Gubányi et al. 2009; Horváth et al. 2012; Horváth and Herczeg 2013; Miklós et al. 2014; Thissen et al. 2015a; Ambros et al. 2016). Understanding the spatio-temporal dynamics and population genetic patterns of this subspecies could be valuable for developing and improving an appropriate and effective conservation action plan and to derive recommendations for the conservation management of this endangered subspecies.

Materials and methods

Study area and samples

Located in the south-western part of Slovakia, the study area lies in the Danubian Plain (Podunajská rovina), part of the Danubian Lowland, belonging to the Pannonian biogeographical region. The landscape was formed by the tributaries of the Danube (Little Danube and others) and by the southern Váh, Nitra and Žitava Rivers. Soil properties and climatic conditions make this region ideal for agriculture. In the area, wheat, sugar beets, sweet corn, vegetables, fruits and tobacco are grown. In the late 19th century, the study area was located in the centre of wet meadows interlaced by meanders of Žitava River, as shown by the Third Military Survey of the Habsburg Empire (1869–1887) (2018). Over the past 130 years, extensive flood protection measures and construction of the channel network fragmented the area of natural meanders of the Žitava River, which changed the direction and hydrodynamics of their flow and led to the aridification of the surrounding ecosystems. By 1926, the original wet meadows had dried up and a network of meanders had formed into a compact wetland habitat crossed by two channels. The consequences of these interventions were still visible in 1964, but the size of wetland habitats had become further reduced by 1990 (Topographic Maps of Czechoslovakia 2018). Today, remnants of the original meander system exist only as a few patches, where the Pannonian root vole still survives. The extension, shape and connectivity of recent wetland habitats scattered across the agricultural landscape are determined by water levels and temporary floods. In the summer and winter of 2010, the extreme precipitation raised water levels and caused the flooding of the Danube (Slovak Hydrometeorological Institute 2011). The last highest water level rise of the river was recorded in spring 2013 (Jakubcová et al. 2014; Matoková and Smrtík 2014), which also influenced our study area. The localities included in this study are situated in the area interlaced by three channels between Patince, Chotín and Marcelová Villages (Fig. 1).

The research was carried out in different types of habitat fragments like waterlogged areas overgrown by *Carex* spp. and *Phragmites* spp. at the edges of channels, oxbow lakes and remnants of former tributaries intersecting large areas of agricultural

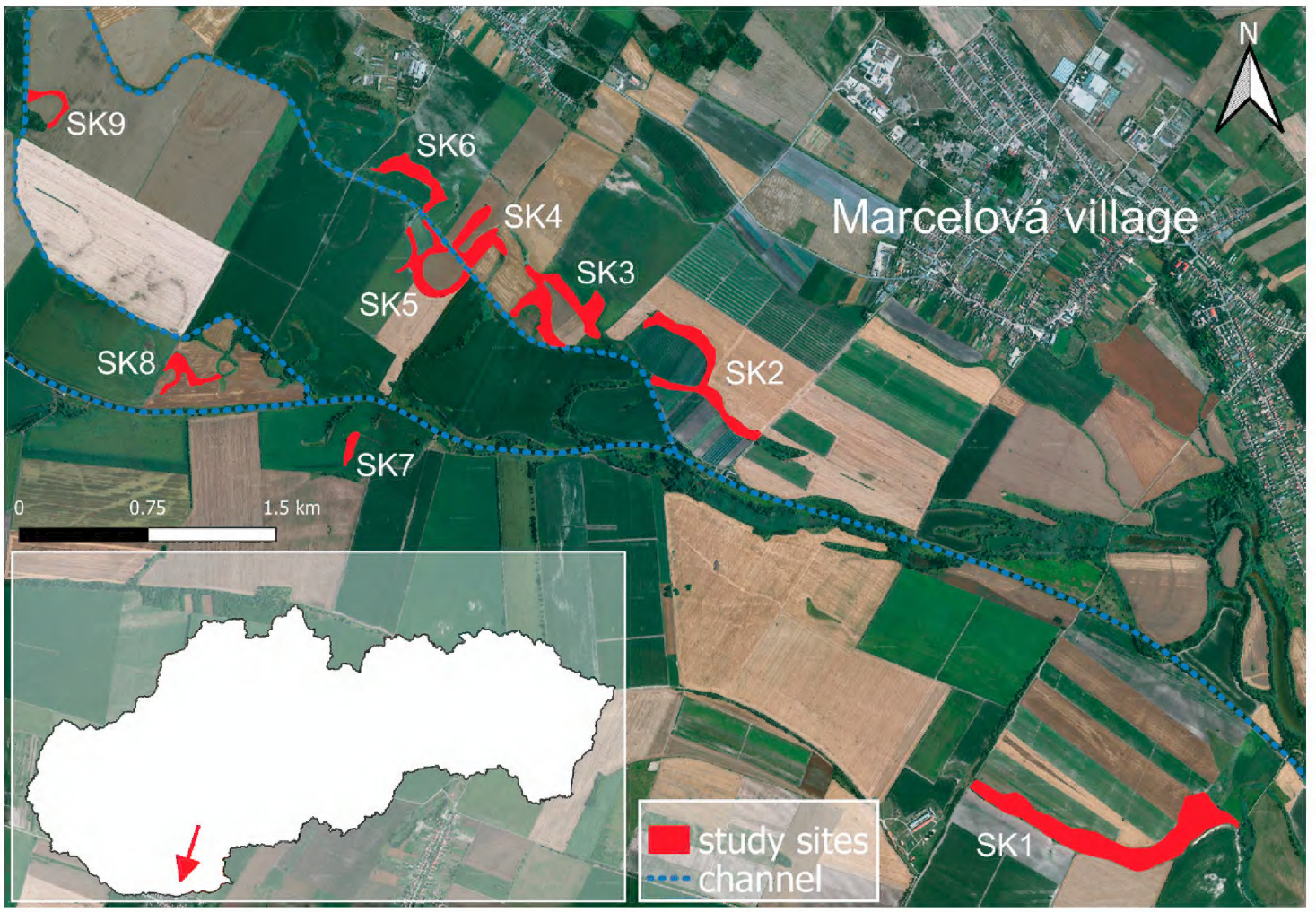

Figure 1. The distribution of the sampled localities of root vole subpopulations in the Danubian Lowland. The sampling sites found in the habitat fragments are indicated by letter and number codes. The insert shows the location of the study area in Slovakia.

lands. Animals were captured with live traps baited with apple and cereals. One line transect of 25 traps with 10 m intervals was established for five consecutive nights in each trapping site. Trapping was conducted three times a year (spring, summer, autumn), from 2014 to 2017. Traps were inspected once a day. Upon capture, each vole was investigated for body weight, age and reproductive status before release. At the first capture, the tail tip of each vole was clipped for genetic analyses. The clipped tail tips were put immediately in 96% ethanol and preserved at -85 °C in the laboratory until DNA extraction. Trapping and sampling methods were realised in agreement with the rules of State Nature Protection of Slovak Republic "Species and habitats monitoring of European importance within the Habitats Directive and the Birds Directive" project. Due to the rarity and endangered status of the Pannonian root vole, sample sizes were low in some trapping transects and, therefore, a total of 69 tissue samples from nine sites were used in molecular analyses.

Changes in habitat fragment patterns and estimation of subpopulation sizes

Five characteristics related to wetland habitat fragments were measured or calculated in six time periods between 2004 and 2019 (January 2004, March 2011, April 2014, March 2017, August 2017 and March 2019): 1) number of all habitat fragments presumably suitable for the Pannonian root vole; 2) number of temporarily

suitable habitat fragments appearing occasionally, depending on water levels; 3) total area of all habitat fragments measured in hectares; 4) average size of habitat fragments (ha); and 5) overall connectivity of our region of interest. Suitable habitat fragments were delimited based on the subspecies' known habitat preference for humid, densely vegetated areas (see Introduction), which markedly differed from the vegetation of agricultural parcels in the study area. The size of particular habitat fragments (ha) was calculated in QGIS software 3.4.12-Madeira (QGIS Development Team 2019). To calculate the connectivity of the whole area (total size: 2873.8 ha), the equivalent connectivity (EC) of PC (probability of connectivity) probabilistic index was used (Saura et al. 2011a, b), where Euclidian edge-to-edge distances with 700 metres' maximum dispersal distance threshold parameter (5% probability for a species to exceed 700 m) were set as a connection between habitat fragments. This distance was set as the average dispersal distance observed on the root vole by Steen (1994). All connectivity indices were calculated in Conefor 2.6 (Saura and Torné 2009). The maps of habitat fragments were created as digitalised topographic maps of Google Earth in map scale 1:5000. Individuals from the same locality were treated as a putative subpopulation (hereafter referred to as subpopulation).

Due to different trapping efforts at each study location, the size of each subpopulation was evaluated as the relative abundance (rA) of individuals captured at the location per 100 trap-nights (C/100TN), based on data without recaptures. The number of captured specimens (N) was recalculated into the transformed rA index assuming a random (Poisson) distribution (rA = (–ln (1-N/100) 100) of small mammals to remove the saturation effect caused by single traps (Caughley 1977). Relative abundance values were averaged over trapping occasions.

We tested the correlation between average rA of Pannonian root vole and average habitat fragment size using Pearson correlation analysis, where both data were log-transformed due to non-normal distribution.

Genetic analysis

DNA extraction

DNA extractions were performed using commercial Isolate II Genomic DNA Kit (Bioline) according to the manufacturer's protocol with the following modifications: during pre-lysis, samples were incubated overnight at room temperature; after adding preheated Elution Buffer G (70 °C), the elution step covered the incubation of samples at room temperature for 30 minutes and after that at 70 °C for 5 minutes before elution.

Analysis of genetic markers and genotyping

The thirteen microsatellite loci included in the analyses were developed for *Microtus arvalis*: Mar003, Mar016, Mar049, Mar063, Mar076 (Walser and Heckel 2008) and for *Alexandromys oeconomus*: Moe1, Moe2, Moe3, Moe4, Moe5, Moe6, Moe7 and Moe8

(Van de Zande et al. 2000). Microsatellite markers were grouped into four multiplex sets (Set1A: Moe1, Moe2, Moe3; Set1B: Moe4, Moe5, Moe6; Set2: Moe7, Moe8; Set3: Mar003, Mar016, Mar049, Mar063, Mar076).

Multiplex PCR reactions were performed in 12 μl volumes containing 2 μl (~80 ng) of DNA and a volume of 10 μl of the following mixture: 3.9 μl of KAPA2G Fast Multiplex Mix (KAPA Biosystems), 0.8 μl of BSA, 0.5 μl (concentration of 10 pm/ng) of each primer and RNase-free water to fill the volume to 10 μl. Amplification of DNA was carried out using peqSTAR 96X Universal thermal cycler (Peqlab).

To amplify microsatellites in Set 1A, Set 1B and Set 2 the PCR reaction consisted of the initial step at 94 °C for 7 minutes, 30 cycles including: denaturation at 94 °C for 1 minute, annealing at 55 °C in case of Set 2 and 60 °C in case of Set 1A and Set 1B for 2 minutes and extension at 72 °C for 90 seconds, followed by a final step at 72 °C for 10 minutes. Microsatellites in Set 3 were amplified in a PCR reaction including the following steps: 7 minutes at 95 °C, 35 cycles of 30 seconds at 94 °C, 1 minute at 57 °C and 90 seconds at 72 °C and after the cycles a final step of 10 minutes at 72 °C. To prepare the genotyping procedure 1 μl PCR product of each sample was mixed with 12 μl formamide and 0.3 μl GeneScan 500-LIZ size standard (Applied Biosystems). After a denaturation step of 5 minutes at 95 °C, a cooling step was implemented. Genotyping was carried out using ABI PRISM 310 Genetic Analyser (Applied Biosystems) and microsatellite genotypes were examined using GeneMapper software v.4.0 (Life Technologies).

Clustering analysis of samples

We successfully genotyped 69 individual samples and the amplification success varied amongst markers (94.2–100%). The presence of null alleles may cause significant heterozygote deficit and deviation from the HWE. We therefore estimated the proportion of null alleles (NA) at each locus in each subpopulation using the programme FREENA (Chapuis and Estoup 2007). All genotypic distributions were in accordance with HWE expectations for all loci and, as we did not detect null alleles based on FREENA, consequently, all loci were included in the analyses.

Bayesian clustering of microsatellite genotypes was performed using STRUCTURE v.2.3.2 (Pritchard et al. 2000). Ten independent runs were performed for each value of K ranging from one to ten under a model assuming admixture and correlated allele frequencies (Falush et al. 2003). Each run comprised a burn-in period of 100,000 replications followed by a run length of 1,000,000 Markov Chain Monte Carlo (MCMC) iterations. The results of replicated runs for each value of K from one to ten were combined using STRUCTURE HARVESTER v.0.6.94 (Earl and vonHoldt 2012) and the optimal value of K was assessed by the inspection of log-likelihood values and according to the ΔK method developed by Evanno et al. (2005). Twenty independent runs were conducted with K fixed at the estimated optimal number of clusters where a burn-in of 100,000 and 1,000,000 MCMC iterations were used. The outputs of replicated runs were combined using the Greedy algorithm in CLUMPP v.1.1.2 (Jakobsson and Rosenberg 2007) and DISTRUCT v.1.1 (Rosenberg 2004) was used to visualise cluster assignments.

Genetic variability in subpopulations

The mean number of alleles (A), observed (H_O) and expected (H_E) heterozygosity (Nei 1978) and inbreeding coefficients (F_{IS}) were calculated using GENETIX v.4.05.2 (Belkhir et al. 2004). The allelic richness (AR) was estimated using the rarefaction procedure for the lowest sample size (n = 10) in the programme FSTAT v.2.9.4 (Goudet 2003). Deviation of subpopulations from Hardy-Weinberg equilibrium (HWE) was calculated with exact tests assessing heterozygote deficiency and excess in GENEPOP v.4.2 (Rousset 2008). The basic level of significance was set to $P = 0.05$ and for multiple comparisons, we applied a Bonferroni procedure compensating for the risk of an inflating type 1 error.

Genetic variation between subpopulations

The programme FREENA was used to estimate global F_{ST}, by performing 10,000 permutations. In addition, a Monte Carlo test of likelihood ratio G-statistic (Goudet et al. 1996) was performed using package *hierfstat* v.0.04-22 (Goudet and Jombart 2015) in R v.3.5.0 (R Core Team 2018) to test the presence of genetic structuring. Estimations of pairwise F_{ST} were implemented in FSTAT v.2.9.4 according to Weir and Cockerham (1984). Significant differences of F_{ST} estimators from zero were tested using 100,000 permutations without the assumption of Hardy-Weinberg equilibrium and significance level was set to $P = 0.05$ and adjusted using the Bonferroni correction. In addition, isolation by distance was tested by assessing the correlation between the geographical distance matrix (given in kilometres) and pairwise $F_{ST}/(1-F_{ST})$ estimates using Mantel's Test with 10,000 permutations in the programme ISOLDE in GENEPOP v.4.2. The GENETIX v.4.05.2 package was used to investigate genetic relationships amongst all genotyped individuals of the nine subpopulations by factorial correspondence analysis (FCA), a method that identifies the linear combination of variables (allele frequencies at different loci) that captures the most variation between observations (individuals or populations) and visualises genetic relationships in 2D space determined by FCA axes. Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) was implemented in GENALEX v.6.5 (Peakall and Smouse 2006, 2012) to test the proportion of genetic variance amongst individuals and subpopulations. Statistical significance of the variance components was assessed with 999 permutations.

Results

Habitat fragment patterns and subpopulation sizes

Between 2004 and 2019, we identified 26 permanent habitat fragments as suitable habitats for root voles, in 14 of which their presence were confirmed. We have also identified several temporarily suitable fragments, the number of which varied season-

ally and annually. All measured characteristics of potential habitats (number of fragments and temporary fragments, total area of all habitat fragments, mean fragment size and connectivity of the whole area) changed over the six time periods of study (see Table 1). In general, connectivity had higher values in periods when the number and size of habitat fragments were higher. In March 2004 and March 2011, the number of temporary fragments increased, filling the matrix of agricultural land between permanent fragments with new suitable habitat (see Fig. 2). The highest values of parameters were observed in 2011, except for the average fragment size. After this period, the total area of habitat fragments, the number of temporary fragments and the connectivity decreased and permanent fragments were thus more isolated. Values of parameters remained relatively similar in the following years. Partial changes in habitat fragment characteristics and connectivity are detectable in seasonal comparison between March and August 2017, with higher values in spring. In a detailed view of the study sites in Fig. 2, it can be seen that, while in August 2017, sites SK3, SK4, SK5 and SK6 were relatively small and isolated from each other, in March 2004, 2011 and 2017, the same sites were larger and only a channel interrupted their direct connection.

The average occupancy of fragmented habitats by the Pannonian root vole varied spatially (Fig. 3). While some fragments had a high relative population size, others had very low. The analysis did not confirm a positive correaltion between average rA of Pannonian root vole and average size of fragmented habitats ($r = 0.15$, $N = 9$, $P = 0.74$).

Clustering analysis

Genetic structuring inferred from STRUCTURE analysis is presented in Fig. 4 for K values ranging from 2 to 5. The ΔK method indicated that the optimal number of clusters was 5. However, this method can evaluate ΔK only for $K > 1$ and the inspection of log-likelihood scores revealed that $K = 1$ had a relatively high likelihood, similar to other values of K. Generally, assignment probabilities were roughly symmetric, which indicated that none of the clustering analyses captured the real population structre. Notably, when K was fixed at 5, SK1 individuals sampled in 2014 (individuals 3, 6, 7 and 8) and an additional individual from SK5 (individual 26 sampled in 2015) had relatively high assignment probabilities to the same cluster and the same for individuals 57 and 60 sampled in SK9 in 2017. However, not all individuals sampled in 2014 and 2015 were assigned to this cluster (indicated with grey in Fig. 4).

Table 1. Changes in patch characteristics during the six time periods between 2004 and 2019.

Time period	Area of all patches (ha)	Mean size of patches (ha)	No. of all patches	No. of temporary patches	Connectivity*
2004 March	179.26	3.51	51	25	840705.8
2011 March	210.19	3.28	64	38	975203.3
2014 April	144.49	3.80	38	12	669245.9
2017 March	149.73	3.56	42	16	679802.1
2017 August	140.92	4.14	34	8	627252.9
2019 March	143.26	4.21	34	8	642355.8

* calculated in a total study area of 2873.8 ha

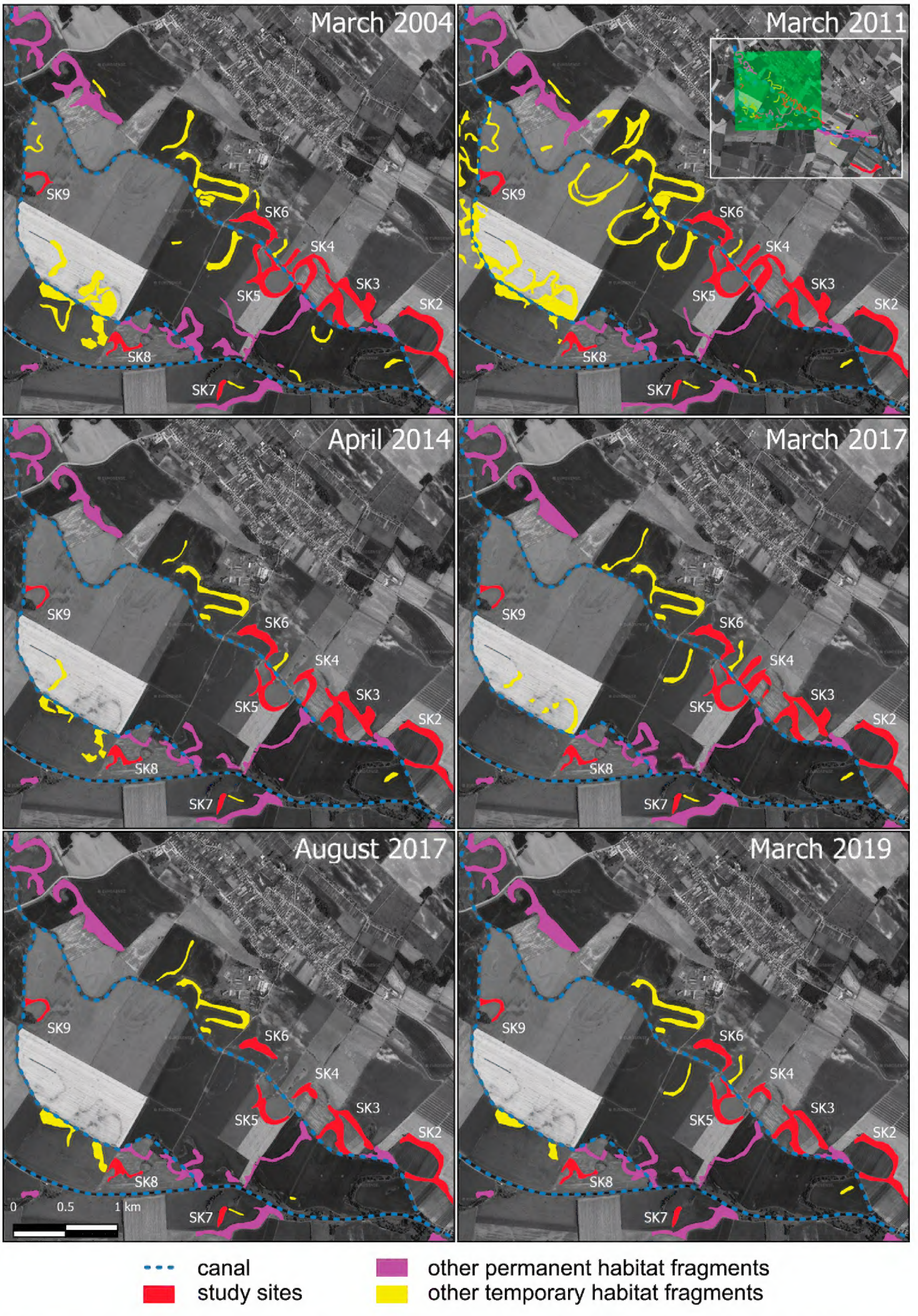

Figure 2. Change in the number of suitable habitat fragments for the Pannonian root vole in the north-western part of the study area over time. The green box in the insert shows the boundaries of the larger maps.

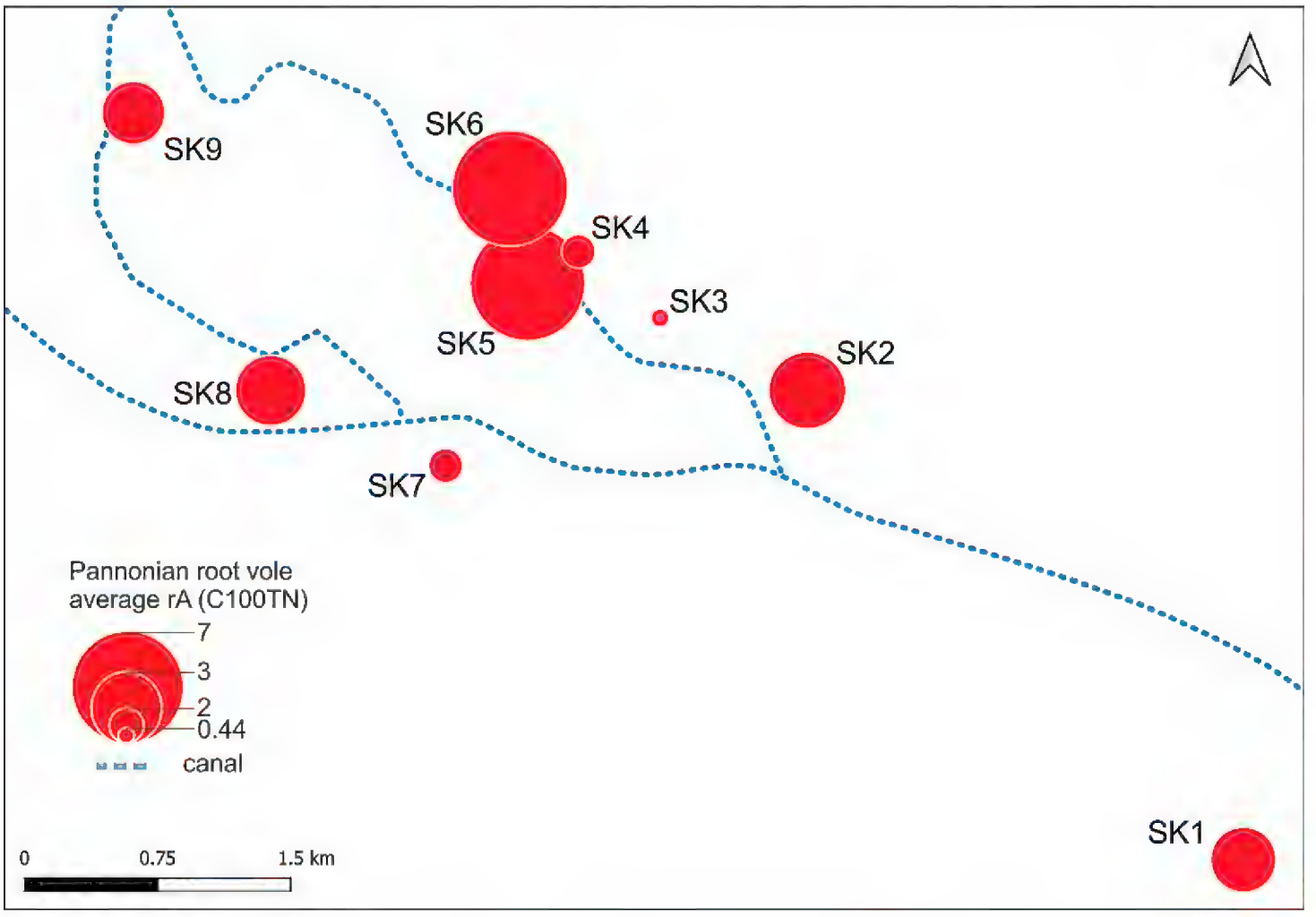

Figure 3. The relative abundance (rA) of Pannonian root vole subpopulations in sampled habitat fragments. Relative abundance values were averaged over trapping occasions and average rA was visualised on the map as graduated symbol size.

Hardy-Weinberg equilibrium and genetic diversity in subpopulations

Clustering analysis did not give a strong evidence of structuring; therefore, measures of genetic diversity were calculated for all subpopulations separately and for all samples pooled together. Genetic diversity and HWE were not calculated for locations SK3, SK4 and SK7 due to their small sample size (N = 3).

The number of alleles per locus in subpopulations ranged from 2 (locus Moe4) to 16 (locus Moe7), and the mean number of alleles per locus (A) ranged from 4.31 to 5.62 (Table 2). Allelic richness (AR) across subpopulations ranged from 4.22 to 5.46, being the highest in the SK1 subpopulation and lowest in the SK8 subpopulation. H_O (range between 0.562 and 0.669) was the highest in subpopulations SK8 and SK9, while the lowest value was shown for SK5. In contrast, H_E (range between 0.624 and 0.657) showed another pattern, with the highest value for SK1 and the lowest value observed for SK8. Two subpopulations, SK8 and SK9 had heterozygosity excess, based on observed and expected heterozygosity values. Subpopulations SK1, SK2, SK5 and SK6 showed significant heterozygote deficiency on the basis of F_{IS} (significant positive values). Subpopulations SK1, SK2 and SK5 deviated from HWE according to heterozygote deficiency exact tests in GENEPOP (P = 0.05) after Bonferroni correction

(Table 2). Heterozygote excess exact tests were not significant in any of the cases (data not shown). Mean number of alleles and expected heterozygosity indicated higher genetic diversity and the exact test showed a significant deficiency of heterozygotes when all samples were pooled together.

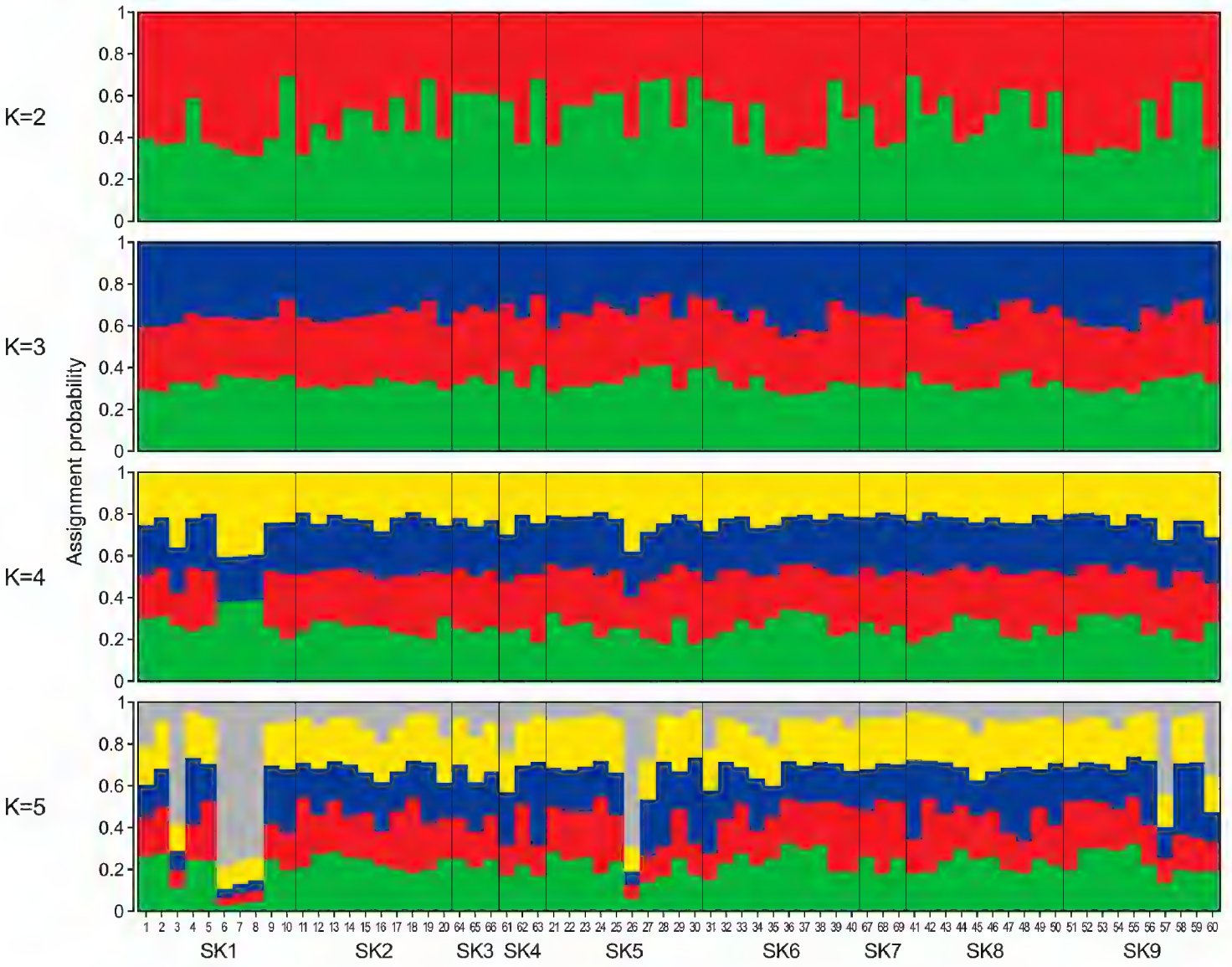

Figure 4. Genetic structure of the sampled root vole subpopulations in the Danubian Lowland. The graph is based on STRUCTURE runs when K was fixed at 2–5. Each individual is represented by a line proportionally divided into colour segments corresponding to its membership in certain clusters. Black lines separate the individuals from different habitat fragments.

Table 2. Genetic diversity in root vole subpopulations and in the total population based on 13 microsatellite loci.

Location	A	AR	H_E	H_O	HWE		F_{IS}
					P	±SE	
SK1	5.62	5.46	0.657	0.586	< 0.001*	0.0000	0.161*
SK2	5.08	5.00	0.654	0.574	< 0.001*	0.0002	0.176*
SK5	4.62	4.49	0.631	0.562	< 0.001*	0.0003	0.161*
SK6	5.15	5.02	0.651	0.619	0.006	0.0010	0.103*
SK8	4.31	4.22	0.624	0.665	0.161	0.0059	-0.013
SK9	4.62	4.50	0.655	0.669	0.228	0.0115	0.030
Total	7.23	7.23	0.694	0.614	< 0.001*	0.0000	0.122*

A: mean number of alleles per locus, *AR*: allelic richness, H_E: expected heterozygosity, H_O: observed heterozygosity, F_{IS}: inbreeding coefficient, *HWE*: *P* values and standard errors of Hardy-Weinberg equilibrium exact tests; * significant values of *HWE* (after Bonferroni correction) and F_{IS}. (In case of the total population, A = AR).

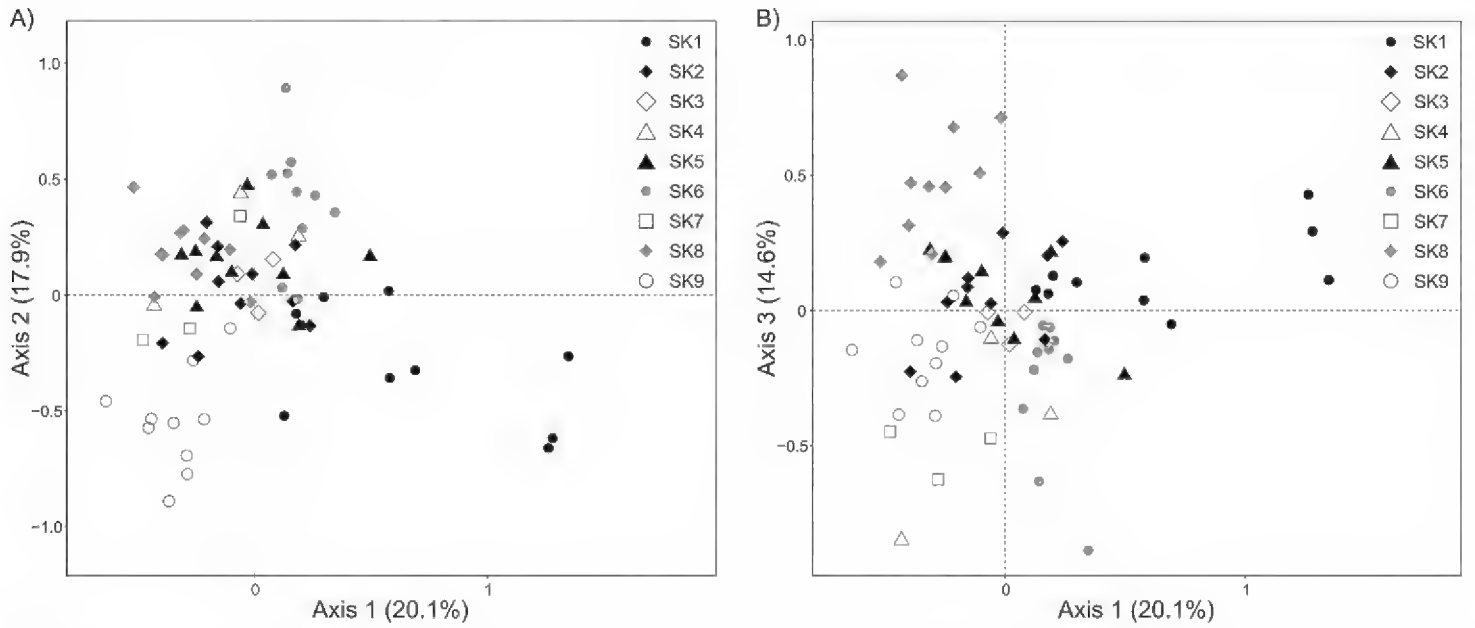

Figure 5. Two-dimensional plots of FCA performed for nine subpopulations showing the 1st and 2nd (**A**) and the 1st and 3rd (**B**) axes. The proportion of explained variance is written in parentheses on each axis.

Table 3. Tests for genetic differentiation between nine root vole subpopulations in the Danubian Lowland. Below diagonal: pairwise F_{ST} values. Above diagonal: *P* values of G-tests implemented in FSTAT.

Location	SK1	SK2	SK5	SK6	SK8	SK9
SK1		0.234	0.307	0.185	< 0.001**	0.010
SK2	0.015		0.344	0.099	0.064	0.020
SK5	0.013	0.004		0.479	0.137	0.012
SK6	0.010	0.009	-0.002		0.001*	< 0.001**
SK8	0.071	0.021	0.016	0.045		< 0.001***
SK9	0.033	0.013	0.014	0.052	0.048	

* Significant *P* values on the nominal level of 0.05 (*), 0.01 (**) and 0.001 (***) after Bonferroni correction.

Genetic differentiation between subpopulations

Subpopulations SK3, SK4 and SK7 were not included in F_{ST} analyses because of their small sample size (N = 3). The global F_{ST} for six subpopulation samples was 0.025 (95% CI: 0.01–0.041). The overall G-test was significant ($P < 0.001$), indicating genetic structuring amongst locations. Pairwise F_{ST} values were relatively low, although variable. The highest F_{ST} values were observed for SK8 and SK9. Pairwise genetic differentiation was not significant in most of the comparisons, except in cases where one subpopulation of the pair was always SK6, SK8 or SK9 (Table 3). Significant F_{ST} was observed between SK1 and SK8, but when the individuals of SK1 sampled in 2014 were excluded from pairwise F_{ST} analyses, this difference became non-significant ($F_{ST} = 0.048$). Microsatellite-based genetic distances did not correlate with geographical distances between subpopulations ($a = 0.0254$, $b \sim 0$, $P = 0.6$), thus spatial separation (IBD) was not confirmed.

The FCA plot, based on individual genotypes, clearly separated SK1 along the first factorial axis (explaining 20.1% of variation) from all other subpopulations. The second axis (explaining 17.9% of variation) mainly separated the individuals from SK1 and SK9, while individuals from SK6 showed only a weak segregation

(Fig. 5A). Subopulation SK8 was separated along the third axis (explaining 14.6% of variation, Fig. 5B).

In the Analysis of Molecular Variance, significant genetic variation was attributed to the differences between subpopulations (4.4%, $P < 0.001$) and most of the variability occured within subpopulations (95.6%).

Discussion

Our results show that genetic variation and differentiation in subpopulations of the Pannonian root vole is in good agreement with connectivity between habitat fragments, with temporary fragments playing an important role in vole migration between flood events.

Habitat connectivity

The number, size and shape of habitat fragments in the studied region varies in time as the result of exogenous factors (precipitation, surface water levels, agricultural activities). These dynamic changes have an effect on fragment connectivity, suggesting that connectivity was positively influenced by the number of habitat fragments. As we have also noted, permanent habitat fragments, relatively distant from each other at one time, can change size and shape and become neighbouring habitats at another time. In addition, the temporary fragments can play the role of stepping stones during vole movements. Thus, despite the constant presence of habitat fragments and channel-side vegetation, fragment connectivity can vary seasonally and yearly, as can change the possibility of individuals' replacement between the studied subpopulations. In Norway, root voles increased dispersal distance as a response to fragmentation, but it was less affected by connectivity (Bjørnstad et al. 1998). Detailed data on the dispersal ability of the Pannonian root vole are still missing, although they have been shown to be able to cross barriers when the quality of original habitats deteriorated and they may follow stepping stones when searching for new habitats (Horváth and Herczeg 2013). Moreover, Kratochvíl and Rosický (1955) observed that Pannonian root voles in sedge (*Carex* sp.) survived on hummocks when the water level increased (50–70 cm). When the water level decreased, voles spread across the lower parts of these hummocks, while some individuals dispersed to new suitable fragments. In view of this, a possible explanation for the observed genetic patterns could be that the animals found shelter on hummocks during floods in 2010 and probably 2013, thus opening the way for admixture. Later, when water levels fell, presumably admixed individuals resettled lower areas.

Genetic differentiation of the Pannonian root vole

Levels of genetic differentiation between the habitat fragments varied, but were mostly non-significant and we found no support for isolation by distance between subpopula-

tions. Bayesian clustering in STRUCTURE did not reveal pronounced genetic structuring, indicated by approximately equal assignment probabilities to different clusters in all cases of K from 2 to 5. This result suggested that dispersal effectively prevents marked genetic subdivision between studied habitat fragments, which can be additionally confirmed by the lack of isolation by distance between fragments. Given the small geographical scale and landscape pattern of the study area, we would expect gene flow between localities to maintain very low or no differentiation between subpopulations. In a study conducted in the Netherlands, Mauritzen et al. (1999) concluded that ditches are likely to favour linear movements of root voles and may enhance connectivity in an agricultural landscape. Most habitat fragments sampled in our study were directly connected by reed- and sedge-lined channels that are likely to serve as dispersal corridors and permanent or temporary habitat fragments in the study area also likely to facilitate dispersal (Ambros et al. 2016). Although the longest geographical distance along the channels separating our sampling sites from their nearest neighbours was nearly 6 km, as for SK1 and SK2, the presence of root vole was confirmed in other permanent habitat fragments between these two sampling sites (which could not be included in our study due to the small number of samples) and most of the habitat fragments sampled were relatively close to each other. Movement distances of male root voles can exceed several hundred metres in a short time or more than two kilometres within a few days (Steen 1994; Andreassen et al. 1996).

Consistent with the changing possibility of individuals' replacement between the studied fragments, AMOVA results also showed a low, but significant, percentage of variability between subpopulations. In addition, signs of genetic differentiation were detected between subpopulations SK8, SK9 and SK6, based on significant pairwise F_{ST} values and the FCA analysis confirmed the separation of these samples. Results may therefore indicate that dispersal is not unhindered between all subpopulations and root vole individuals in the network of studied habitat fragments may not be viewed as a panmictic population.

Genetic diversity of the Pannonian root vole

In a detailed study, Hulejová Sládkovičová et al. (2018) already noted that, despite the high genetic variability of the subspecies, local populations of Pannonian root vole may show signs of genetic depletion. Although our analyses were limited by small sample sizes due to the rarity of the Pannonian root vole, our results indicate lower genetic diversity in subpopulations (in terms of allelic richness, mean number of alleles per locus and expected heterozygosity) than what was measured in other populations from south-western Slovakia (Hulejová Sládkovičová et al. 2018). Similarly, reduced genetic diversity (based on the mean number of alleles per locus and the expected heterozygosity) was observed compared to the populations of another isolated Western European root vole subspecies, namely *A. o. arenicola* (Van de Zande et al. 2000). Expected heterozygosity and mean number of alleles in the total studied population indicated decreased variability compared to other Slovakian populations (Hulejová Sládkovičová et al. 2018). Low genetic diversity of the studied root vole

population putatively reflects a pronounced geographical isolation of the surviving population and the decrease in effective population size that has likely occurred. In addition, flood events may have reduced the size of the vole population (Kratochvíl and Rosický 1955), which may have created a bottleneck, although its influence on the genetic diversity of the studied population cannot be clearly confirmed on the basis of our results alone. The reduced genetic diversity is in accordance with the view of Hulejová Sládkovičová et al. (2018) who suggested that the Danube River and associated branch network could facilitate vole dispersal, but local populations embedded in the agricultural landscape and further from the Danube are likely to remain isolated. Moreover, based on the evaluation of the distribution of Pannonian root vole, our study area is close to the eastern boundary of the subspecies' range (Gubányi et al. 2009; Ambros et al. 2016) and populations occurring here are likely isolated by the distance from other populations to the west.

Subpopulations SK8 and SK9 tend to have lower levels of allelic richness, which is consistent with the possibly lower probability of dispersal through the agricultural land matrix compared to other sites. However, we did not find significant deviation from the Hardy-Weinberg equilibrium in these subpopulations. We observed deviations from the Hardy-Weinberg equilibrium in SK1, SK2 and SK5 and significant positive F_{IS} coefficients in the same subpopulations, together with SK6, which may result from the social structure of root voles. Matriline-based groups in root vole populations (Tast 1966) give the possibility of sampling kin individuals, which may result in departures from the Hardy-Weinberg equilibrium (Aars et al. 2006). On the other hand, Stewart et al. (1999) argue that genetic composition (and departures from the Hardy-Weinberg equilibrium) of water vole (*Arvicola amphibius*) colonies is under the influence of yearly fluctuation and suggested that genetic drift is a plausible cause resulting in that pattern. They interpreted high levels of genetic diversity as a sign of gene flow between colonies. Similarly, despite the relatively large number of analysed individuals, Pilot et al. (2010) observed yearly changes in departures from the Hardy-Weinberg equilibrium in a root vole population in Poland; nevertheless, a high level of genetic diversity was maintained over the years. In addition, they found no genetic differentiation between years regardless of changes in density, kin structure and deviations from HWE, indicating that genetic composition is stable over time in large, demographically-stable populations. In contrast, studies of water vole metapopulations revealed temporal genetic differentiation, but genetic variability remained high as a result of gene flow (Stewart et al. 1999; Aars et al. 2006).

Possible migration from Danube River

Regarding individuals 3, 6, 7 and 8 (sampled in 2014 and 2015) in SK1, highlighted by clustering analyses, their high assignment probability to a separate cluster may reflect their distinct origin. Habitat fragment SK1 is the closest to the Danube River amongst the studied fragments and the floods in 2010 or 2013 potentially facilitated dispersal from further areas and the aforementioned individuals might be immigrants or their

descendants. Other individuals that had relatively high assignment probabilities to the same cluster were captured in SK5 (individual 26 from 2015) and SK9 (individual 57 and 60). These can be found at a few kilometres distance from SK1, but given the small spatial scale, it is not unlikely that these specimens may be the offspring of dispersing individuals. Alternatively, it is also possible that genetic drift over time changed the genetic composition of subpopulations in the fragmented landscape; hence, some (but not all) samples collected in 2014 and 2015 were highlighted by STRUCTURE clustering. However, we are not able to declare which possibility is more plausible without genotyping individuals from other areas and without temporal analysis of samples.

The genetic diversity of the local subpopulations in the study area is relatively low; although the studied subpopulations probably have connections with each other due to the effect of extensive floods and the network of fragments and channels in the agricultural landscape, their reduced genetic variability is detectable compared to the pooled genetic variability of other populations of Pannonian root vole occurring closer to the more uninterrupted marshlands in Szigetköz, Hanság and Neusiedlersee Regions (Hulejová Sládkovičová et al. 2018). Based on the pattern of remnant habitat fragments, it is likely that root vole subpopulations in our study area form a metapopulation system and the observed genetic patterns do not contradict this possibility. However, studies on extinction and recolonisation patterns and migration rates would be essential to support this view (Gaggiotti 2004; van der Merwe et al. 2016).

Conclusions and management implications for conservation

Landscape changes and habitat destruction resulted in the fragmented distribution of root vole habitats in the study area and fluctuating surface water levels induce considerable changes in habitat size, quality and connectivity to this day. Only one fragment (SK2) in our study area is protected as a Special Protection Area. However, for the long-term persistence of root vole populations, it would be critical to ensure legal protection of habitats. The importance of protected core areas has been demonstrated for water vole metapopulations (MacPherson and Bright 2011) and mainland or source population demography has been shown to shape habitat use of root voles (Glorvigen et al. 2013). Based on the low differentiation, it seems unlikely that the studied vole subpopulations formed a mainland-island type metapopulation system (Stewart et al. 1999), but the signs of admixture highlight the vital role of habitat fragments and gene flow in the maintenance of genetic diversity. On the other hand, Van de Zande et al. (2000) proposed that weak differentiation may be an indicator of progressive isolation of local populations and accordingly, the prevention of fragmentation should be considered in the conservation management of *A. o. arenicola*. It has been suggested for other vole species and subspecies that persistent gene flow between habitat fragments is crucial for the maintenance of genetic variability (Telfer et al. 2003). Therefore, it is necessary to protect not only habitat fragments with current occurrences of the Pannonian root vole, but also other potentially-suitable habitat fragments, to form a habitat network and to ensure the possibility of gene flow (Neuwald 2010). Water levels in

the study area and, as we demonstrated, total size of permanent habitat fragments and number of temporary habitats are in dynamic change, which further emphasises the contribution of these fragments to the survival of the subspecies in the agricultural landscape (Kalmár and Riezing 2017).

It is expected that the overall genetic diversity of the subpopulations will decrease as a result of their small size and isolated location in the agricultural matrix. This implies that the restoration of habitats and corridors is indispensable for the long-term preservation of diversity, as has been stressed earlier (Thissen et al. 2015a; Hulejová Sládkovičová et al. 2018). As a habitat specialist, the Pannonian root vole is sensitive to habitat quality changes, which are likely to be the key factors determining habitat use (Glorvigen et al. 2013; Horváth and Herczeg 2013). Thus, it is important to preserve optimal water level conditions for the root vole, for example, by controlled construction of infrastructure that may cause aridification and the disappearance of the subspecies (List et al. 2010; Kalivodová et al. 2018). For this reason, it is necessary to take the needs of the subspecies into consideration when infrastructural projects are planned and managers should be involved in landscape and land-use planning. Finally, long-term monitoring is recommended to provide further information about population and subpopulation parameters (Hayes et al. 2017), genetic diversity and structure (Proença-Ferreira et al. 2019) and on the changes of habitats (Martensen et al. 2017) to elaborate an effective conservation management action plan that ensures the long-term survival of the Pannonian root vole.

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References

Aars J, Dallas JF, Piertne SB, Marshall F, Gow JL, Telfer S, Lambin X (2006) Widespread gene flow and high genetic variability in populations of water voles *Arvicola terrestris* in

patchy habitats. Molecular Ecology 15(6): 1455–1466. https://doi.org/10.1111/j.1365-294X.2006.02889.x

Ambros M, Baláz I, Klimant P, Tulis F, Dudich A, Stollmann A, Horváth G (2016) The occurrence of Pannonian root vole (*Microtus oeconomus mehelyi*) in small mammals' communities in Danubian Plain. Folia Oecologica 43(1): 83–88.

Andreassen HP, Halle S, Ims RA (1996) Optimal width of movement corridors for root voles: Not too narrow and not too wide. Journal of Applied Ecology 33(1): 63–70. https://doi.org/10.2307/2405016

Baláž I, Fraňová S (2013) Biometric values comparison of somatic and cranial features of two *Microtus oeconomus* subspecies. Folia Faunistica Slovaca 18(1): 59–66.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France). https://kimura.univ-montp2.fr/genetix/

Bjørnstad ON, Andreassen HP, Ims RA (1998) Effects of habitat patchiness and connectivity on the spatial ecology of the root vole *Microtus oeconomus*. Journal of Animal Ecology 67(1): 127–140. https://doi.org/10.1046/j.1365-2656.1998.00175.x

Brunhoff C, Galbreath KE, Fedorov VB, Cook JA, Jaarola M (2003) Holarctic phylogeography of the root vole (*Microtus oeconomus*): Implications for late Quaternary biogeography of high latitudes. Molecular Ecology 12(4): 957–968. https://doi.org/10.1046/j.1365-294X.2003.01796.x

Buzan EV, Krystufek B, Bryja J (2010) Microsatellite markers confirm extensive population fragmentation of the endangered Balkan palaeoendemic Martino's vole (*Dinaromys bogdanovi*). Conservation Genetics 11(5): 1783–1794. https://doi.org/10.1007/s10592-010-0071-2

Caughley G (1977) Analysis of Vertebrate Populations. Wiley, New York, 234 pp.

Centeno-Cuadros A, Roman J, Delibes M, Godoy JA (2011) Prisoners in their habitat? Generalist dispersal by habitat specialists: A case study in southern water vole (*Arvicola sapidus*). PLoS ONE 6(9): e24613. https://doi.org/10.1371/journal.pone.0024613

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24(3): 621–631. https://doi.org/10.1093/molbev/msl191

Čížková H, Květ J, Comin FA, Laiho R, Pokorný J, Pithart D (2013) Actual state of European wetlands and their possible future in the context of global climate change. Aquatic Sciences 75(1): 3–26. https://doi.org/10.1007/s00027-011-0233-4

Council of Europe (1982) Convention on the Conservation of European Wildlife and Natural Habitats. Official Journal of the European Communities L 38: 3–32. http://data.europa.eu/eli/convention/1982/72/oj

de Sélys Longchamps ME (1841) Note sur le Mus agrestis de Linné. Bulletins de l'Académie royale des sciences et belles-lettres de Bruxelles 8(2): 234–237.

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4(2): 359–361. https://doi.org/10.1007/s12686-011-9548-7

Éhik Gy (1928) Néhány adat Magyarország emlősfaunájának ismeretéhez. Annales Historico-Naturales Musei Nationalis Hungarici 25: 195–203.

European Commission (1992) Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna And Flora. Official Journal of the European Union L 206: 7–50. http://data.europa.eu/eli/dir/1992/43/2013-07-01

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14(8): 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131(2): 479–491. https://doi.org/10.1093/genetics/131.2.479

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164(4): 1567–1587.

Fietz J, Tomiuk J, Loeschcke V, Weis-Dootz T, Segelbacher G (2014) Genetic consequences of forest fragmentation for a highly specialized arboreal mammal-the edible dormouse. PLoS ONE 9(2): e88092. https://doi.org/10.1371/journal.pone.0088092

Gaggiotti OE (2004) Multilocus genotype methods for the study of metapopulation processes. In: Hanski I, Gaggiotti OE (Eds) Ecology, Genetics, and Evolution of Metapopulations. Elsevier Academic Press, London, 367–386. https://doi.org/10.1016/B978-012323448-3/50017-9

Gaines MS, Diffendorfer JE, Tamarin RH, Whittam TS (1997) The effects of habitat fragmentation on the genetic structure of small mammal populations. The Journal of Heredity 88(4): 294–304. https://doi.org/10.1093/oxfordjournals.jhered.a023107

Glorvigen P, Andreassen HP, Ims RA (2013) Local and regional determinants of colonisation-extinction dynamics of a riparian mainland-island root vole metapopulation. PLoS ONE 8(2): e56462. https://doi.org/10.1371/journal.pone.0056462

Goudet J (2003) FSTAT (ver. 2.9.4): a program to estimate and test population genetics parameters. Updated from Goudet (1995). https://www2.unil.ch/popgen/softwares/fstat.htm

Goudet J, Jombart T (2015) hierfstat: estimation and tests of hierarchical F-statistics. R package version 0.04-22, 10. https://CRAN.R-project.org/package=hierfstat

Goudet J, Raymond M, de Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144(4): 1933–1940. https://doi.org/10.1093/genetics/144.4.1933

Gubányi A, Dudich A, Stollmann A, Ambros M (2009) Distribution and conservation management of the root vole (*Microtus oeconomus*) populations along the Danube in Central Europe (Rodentia: Arvicolinae). Lynx 40: 29–42.

Hayes LD, Ebensperger LA, Kelt DA, Meserve PL, Pillay N, Viblanc VA, Schradin C (2017) Long-term field studies on rodents. Journal of Mammalogy 98(3): 642–651. https://doi.org/10.1093/jmammal/gyw180

Horváth GF, Herczeg R (2013) Site occupancy response to natural and anthropogenic disturbances of root vole: Conservation problem of a vulnerable relict subspecies. Journal for Nature Conservation 21(5): 350–358. https://doi.org/10.1016/j.jnc.2013.03.004

Horváth GF, Horvath B, Sali N, Herczeg R (2012) Community-level response to different human disturbances and land use of small mammals in two marshland habitat patches in Hungary. Archives of Biological Sciences 64(2): 613–628. https://doi.org/10.2298/ABS1202613H

Hulejová Sládkovičová V, Miklós P, Hollá K, Búliková V, Žiak D (2016) Habitat preferences of small mammals occurring in two studied wetlands of Žitný ostrov area. Folia Faunistica Slovaca 21(3): 229–233.

Hulejová Sládkovičová V, Dąbrowski MJ, Žiak D, Miklós P, Gubányi A, La Haye MJ, Bekker D, Thissen J, Herzig-Straschil B, Kocian Ľ, Gliwicz J (2018) Genetic variability of the cold-tolerant *Microtus oeconomus* subspecies left behind retreating glaciers. Mammalian Biology 88(1): 85–93. https://doi.org/10.1016/j.mambio.2017.11.007

Jakobsson M, Rosenberg NA (2007) CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics (Oxford, England) 23(14): 1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Jakubcová A, Grežo H, Hreško J (2014) Identification of areas with significant flood risk at the confluence of Danube and Ipeľ rivers (southern Slovakia). Natural Hazards 75(1): 849–867. https://doi.org/10.1007/s11069-014-1354-6

Kalivodová M, Kanka R, Miklós P, Hulejová Sládkovičová V, Žiak D (2018) Importance of wetland refugia in agricultural landscape provided based on the community characteristics of small terrestrial mammals. Ekologia (Bratislava) 37(4): 358–368. https://doi.org/10.2478/eko-2018-0027

Kalmár S, Riezing N (2017) Az északi pocok (*Microtus oeconomus mehelyi*) élőhelyválasztása és állományainak vizsgálata Győr-Moson-Sopron megyében. In: Takács G, Pellinger A (Eds) Rence (Monograph Series of Fertő-Hanság National Park Directorate): Vol.2. Researches of Fertő-Hanság National Park I. Monograph. Fertő-Hanság National Park Directorate, Sarród, 76–97.

Kozakiewicz M, Gortat T, Kozakiewicz A, Barkowska M (1999) Effects of habitat fragmentation on four rodent species in a Polish farm landscape. Landscape Ecology 14(4): 391–400. https://doi.org/10.1023/A:1008070610187

Kratochvíl J, Rosický B (1955) Hraboš severní (*Microtus oeconomus*) relikt zvířeny z doby ledové v ČSR. Práce Brněnské základny ČSAV 24(1): 33–72.

Lanszki J, Rozner G, Széles G (2015) A Pogány-völgyi rétek Natura 2000 terület kisemlős közösségeinek vizsgálata, különös tekintettel az északi pocok (*Microtus oeconomus*) előfordulására. Natura Somogyiensis 27: 107–114.

Lino A, Fonseca C, Rojas D, Fischer E, Pereira MJR (2019) A meta-analysis of the effects of habitat loss and fragmentation on genetic diversity in mammals. Mammalian Biology 94(1): 69–76. https://doi.org/10.1016/j.mambio.2018.09.006

Linzey AV, Shar S, Lkhagvasuren D, Juškaitis R, Sheftel B, Meinig H, Amori G, Henttonen H (2016) *Microtus oeconomus* (errata version published in 2017). The IUCN Red List of Threatened Species 2016: e.T13451A115113894. https://doi.org/10.2305/IUCN.UK.2016-3.RLTS.T13451A22347188.en

List R, Pergams OR, Pacheco J, Cruzado J, Ceballos G (2010) Genetic divergence of *Microtus pennsylvanicus chihuahuensis* and conservation implications of marginal population extinctions. Journal of Mammalogy 91(5): 1093–1101. https://doi.org/10.1644/09-MAMM-A-168.1

MacPherson JL, Bright PW (2011) Metapopulation dynamics and a landscape approach to conservation of lowland water voles (*Arvicola amphibius*). Landscape Ecology 26(10): 1395–1404. https://doi.org/10.1007/s10980-011-9669-0

Marchi C, Andersen LW, Damgaard C, Olsen K, Jensen TS, Loeschcke V (2013) Gene flow and population structure of a common agricultural wild species (*Microtus agrestis*) under different land management regimes. Heredity 111(6): 486–494. https://doi.org/10.1038/hdy.2013.70

Martensen AC, Saura S, Fortin MJ (2017) Spatio-temporal connectivity: Assessing the amount of reachable habitat in dynamic landscapes. Methods in Ecology and Evolution 8(10): 1253–1264. https://doi.org/10.1111/2041-210X.12799

Matoková K, Smrtík P (2014) Dunaj opäť ukázal svoju silu. *Deň Dunaja*, Bratislava, Slovakia, June 2014. http://www.shmu.sk/File/Hydrologia/Publikacna_cinnost/2014/2014_DenDunaja_Matokova_Smrtnik_Dunaj%20opat%20ukazal%20svoju%20silu.pdf

Mauritzen M, Bergers PJ, Andreassen HP, Bussink H, Barendse R (1999) Root vole movement patterns: Do ditches function as habitat corridors? Journal of Applied Ecology 36(3): 409–421. https://doi.org/10.1046/j.1365-2664.1999.00414.x

Miklós P, Žiak D, Ambros M, Stollmann A (2011) Nové nálezy hraboša severského panónskeho (*Microtus oeconomus mehelyi*) v oblasti Podunajskej roviny. Folia Faunistica Slovaca 16(1): 19–23.

Miklós P, Žiak D, Ambros M, Dudich A, Stollmann A, Hulejová Sládkovičová V (2014) Records of Pannonian root vole (*Microtus oeconomus mehelyi*) in the south-western Slovakia in 2011. Folia Faunistica Slovaca 19(1): 57–63.

Mortelliti A, Amori G, Capizzi D, Rondinini C, Boitani L (2010) Experimental design and taxonomic scope of fragmentation studies on European mammals: Current status and future priorities. Mammal Review 40(2): 125–154. https://doi.org/10.1111/j.1365-2907.2009.00157.x

Musser GG, Carleton MD (2005) Superfamily Muroidea. In: Wilson DE, Reeder DM (Eds) Mammal Species of the World (3rd ed.). Johns Hopkins University Press, Baltimore, 894–1531.

Nehring A (1899) Über das Vorkommen der nordischen Wühlratte (*Aruicola ratticeps* Keys, u. Blas.) bei Brandenburg an der Havel und bei Anklam in Vorpommern und in Ostpreussen. Sitzungsber. d. Gesellsch. naturforsch. Freunde zu Berlin 3: 57–59; 4: 67–71.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89(3): 583–590.

Neuwald JL (2010) Population isolation exacerbates conservation genetic concerns in the endangered Amargosa vole, *Microtus californicus scirpensis*. Biological Conservation 143(9): 2028–2038. https://doi.org/10.1016/j.biocon.2010.05.007

Pardiñas UFJ, Myers P, León-Paniagua L, Ordóñez Garza N, Cook JA, Kryštufek B, Haslauer R, Bradley R, Shenbrot G, Patton JL (2017) Family Cricetidae (True Hamsters, Voles, Lemmings and New World Rats and Mice). In: Wilson DE, Lacher Jr TE, Mittermeier RA (Eds) Handbook of the Mammals of the World (Vol. 7). Rodents II. Lynx Edicions, Barcelona, 204–279.

Parmenter BE, Lim MC, Chen Y, Conroy CJ, Olechnowski BF (2015) Microsatellite variation in the Owens Valley Vole (*Microtus californicus vallicola*). The Southwestern Naturalist 60(2–3): 256–262. https://doi.org/10.1894/SWNAT-D-14-0004.1

Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1): 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics (Oxford, England) 28(19): 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pilot M, Dąbrowski MJ, Jancewicz E, Schtickzelle N, Gliwicz J (2010) Temporally stable genetic variability and dynamic kinship structure in a fluctuating population of the root vole *Microtus oeconomus*. Molecular Ecology 19(13): 2800–2812. https://doi.org/10.1111/j.1365-294X.2010.04692.x

Pita R, Mira A, Beja P (2010) Spatial segregation of two vole species (*Arvicola sapidus* and *Microtus cabrerae*) within habitat patches in a highly fragmented farmland landscape. European Journal of Wildlife Research 56(4): 651–662. https://doi.org/10.1007/s10344-009-0360-6

Pita R, Mira A, Beja P (2014) *Microtus cabrerae* (Rodentia: Cricetidae). Mammalian Species 46(912): 48–70. https://doi.org/10.1644/912.1

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155(2): 945–959.

Proença-Ferreira A, Ferreira C, Leitão I, Paupério J, Sabino-Marques H, Barbosa S, Lambin X, Alves PC, Beja P, Moreira F, Mira A, Pita R (2019) Drivers of survival in a small mammal of conservation concern: An assessment using extensive genetic non-invasive sampling in fragmented farmland. Biological Conservation 230: 131–140. https://doi.org/10.1016/j.biocon.2018.12.021

Purger JJ (2014) Survey of the small mammal fauna in north-western Somogy county (Hungary), based on barn owl *Tyto alba* (Scopoli, 1769) pellet analysis. Natura Somogyiensis 24: 293–304.

QGIS Development Team (2019) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Redeker S, Andersen LW, Pertoldi C, Madsen AB, Jensen TS, Jørgensen JM (2006) Genetic structure, habitat fragmentation and bottlenecks in Danish bank voles (*Clethrionomys glareolus*). Mammalian Biology 71(3): 144–158. https://doi.org/10.1016/j.mambio.2005.12.003

Rosenberg NA (2004) DISTRUCT: A program for the graphical display of population structure. Molecular Ecology Notes 4(1): 137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Rousset F (2008) genepop'007: A complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8(1): 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Rushton SP, Barreto GW, Cormack RM, Macdonald DW, Fuller R (2000) Modelling the effects of mink and habitat fragmentation on the water vole. Journal of Applied Ecology 37(3): 475–490. https://doi.org/10.1046/j.1365-2664.2000.00504.x

Saura S, Torné J (2009) Conefor Sensinode 2.2: A software package for quantifying the importance of habitat patches for landscape connectivity. Environmental Modelling & Software 24(1): 135–139. https://doi.org/10.1016/j.envsoft.2008.05.005

Saura S, Estreguil C, Mouton C, Rodríguez-Freire M (2011a) Network analysis to assess landscape connectivity trends: Application to European forests (1990–2000). Ecological Indicators 11(2): 407–416. https://doi.org/10.1016/j.ecolind.2010.06.011

Saura S, González-Ávila S, Elena-Rosselló R (2011b) Evaluación de los cambios en laconectividad de los bosques: El índice del área conexa equivalente y su aplicación a los bosques de Castilla y León. Revista Montes 106(3): 15–21.

Slovak Hydrometeorological Institute (2011) Flood report 2010. http://www.shmu.sk/File/HIPS/Povodnova_sprava_2010.pdf

Steen H (1994) Low survival of long distance dispersers of the root vole (*Microtus oeconomus*). Annales Zoologici Fennici 31: 271–274.

Stewart WA, Dallas JF, Piertney SB, Marshall F, Lambin X, Telfer S (1999) Metapopulation genetic structure in the water vole, *Arvicola terrestris*, in NE Scotland. Biological Journal of the Linnean Society. Linnean Society of London 68(1–2): 159–171. https://doi.org/10.1111/j.1095-8312.1999.tb01164.x

Stollmann A, Ambros M (1998) Hraboš severský panónsky (*Microtus oeconomus* Pallas, 1776 ssp. Méhelyi Éhik, 1958)–kriticky ohrozený druh cicavca na Slovensku. In: Urban P (Ed.) Výskum a Ochrana Cicavcov na Slovensku. Banská Bystrica, 119–126.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: Where are we now? Molecular Ecology 19(17): 3496–3514. https://doi.org/10.1111/j.1365-294X.2010.04691.x

Tast J (1966) The root vole, *Microtus oeconomus* (Pallas), as an inhabitant of seasonally flooded land. Annales Zoologici Fennici 3: 127–171.

Telfer S, Dallas JF, Aars J, Piertney SB, Stewart WA, Lambin X (2003) Demographic and genetic structure of fossorial water voles (*Arvicola terrestris*) on Scottish islands. Journal of Zoology 259(1): 23–29. https://doi.org/10.1017/S0952836902003321

Third Military Survey of the Habsburg Empire (1869–1887) (2018) Third Military Survey of the Habsburg Empire. https://mapire.eu/en/map/thirdsurvey25000/

Thissen JB, Bekker DL, Spreitzer K, Herzig-Straschil B (2015a) The distribution of the Pannonic root vole (*Microtus oeconomus mehelyi* Ehik, 1928) in Austria. Lutra 58(1): 3–22.

Thissen JB, Bekker DL, Herzig-Straschil B, Koelman RM (2015b) Two new records of the Pannonic root vole (*Microtus oeconomus mehelyi* Ehik, 1928) in Austria. Lutra 58(2): 127–131.

Topographic Maps of Czechoslovakia (2018) Topographic Maps of Czechoslovakia. https://www.staremapy.sk/

Van de Zande L, Van Apeldoorn RC, Blijdenstein AF, De Jong D, Van Delden W, Bijlsma R (2000) Microsatellite analysis of population structure and genetic differentiation within and between populations of the root vole, *Microtus oeconomus* in the Netherlands. Molecular Ecology 9(10): 1651–1656. https://doi.org/10.1046/j.1365-294x.2000.01051.x

van der Merwe J, Hellgren EC, Schauber EM (2016) Variation in metapopulation dynamics of a wetland mammal: The effect of hydrology. Ecosphere 7(3): e01275. https://doi.org/10.1002/ecs2.1275

Walser B, Heckel G (2008) Microsatellite markers for the common vole (*Microtus arvalis*) and their cross-species utility. Conservation Genetics 9(2): 479–481. https://doi.org/10.1007/s10592-007-9355-6

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution; International Journal of Organic Evolution 38(6): 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Willi Y, Hoffmann AA (2009) Demographic factors and genetic variation influence population persistence under environmental change. Journal of Evolutionary Biology 22(1): 124–133. https://doi.org/10.1111/j.1420-9101.2008.01631.x

Wilson MC, Chen XY, Corlett RT, Didham RK, Ding P, Holt RD, Holyoak M, Hu G, Hughes AC, Jiang L, Laurance WF, Liu J, Pimm SL, Robinson SK, Russo SE, Si X, Wilcove DS, Wu J, Yu M (2016) Habitat fragmentation and biodiversity conservation: Key findings and future challenges. Landscape Ecology 31(2): 219–227. https://doi.org/10.1007/s10980-015-0312-3